REMARKS

Claims 4 and 7 have been amended *supra* to reflect that the RIAA and IAA of the invention are required to be in a synergistic ratio. Support for these amendments may be found throughout the Specification and Examples, most particularly in Example 4, Tables 5 and 6. Claims 1-3 have been cancelled without prejudice. The Applicants aver that the amendments to the claims as presented *supra* do not represent new matter and respectfully request entry of the claims as amended.

Claim Rejections – 35 U.S.C. § 102

Claims 1-3 stand rejected under 35 U.S.C. § 102(e) as being anticipated by Shahlal et al. (US 6,583,322, PTO-892). The Applicants have cancelled Claims 1-3 thereby rendering this rejection moot. As such, the Applicants respectfully request withdrawal of this rejection.

Claim Rejections – 35 U.S.C. § 103

Claims 4-7 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Tobe (US 5,604,263, PTO-892; hereinafter "Tobe") for obviousness. The Applicants respectfully disagree with this assessment.

The Examiner appears to maintain in the third paragraph on page 6 of the Action that

> *"...because Tobe teaches that compounds which has structure similar to that of prostaglandin E2 are useful in treating PGE2 mediated inflammation, and reduced isoalpha acid has a structure similar to that of prostaglandin E2, and further reduced isoalpha acid is a derivative of isoalpha acid which is used for treating inflammatory disorder. Thus, one of ordinary skill in the art at the time of the invention would have been motivated to employ reduced isoalpha acid such as dihydro-isohumulone in combination with isoalpha acid with reasonable expectation of treating PGE2 mediated inflammation."*

The Applicants respectfully disagree.

The Applicants maintain that one of skill in the pharmaceutical arts will recognize that mere structural similarity between compounds is insufficient to presume similar biologic activities. Furthermore, the Examiner's inference that the reduced form of a compound, here reduced isoalpha acids, should have similar biologic activity to the non reduced parent compound (here isoalpha acids), is belied too often in medicinal chemistry to be taken as obvious. This may be illustrated by the following example.

Consider the difference between salicylic acid its reduced congeners salicylaldehyde and salicylic alcohol. Salicylic acid is a well-known and widely used NSAID (non-steroidal anti0inflammatory drug). However upon reduction (using an appropriate metal hydride e.g., LiAlH4 or NaBH4) the resulting reduced products, salicylic alcohol & salicylaldehyde, are potent allergens. The Applicants maintain that this example fully illustrates both that compounds of similar structure or compounds derived from reduction of a parent compound need not have similar biologic activities and as such are not obvious. The Examiner's attention is directed to Contact Dermatitis, Vol. 52 Issue 2 Page 93 February 2005, Allergic contact dermatitis from salicyl alcohol and salicylaldehyde in aspen bark (Populus tremula) Kristiina Aalto-Korte, Jarmo Välimaa, Maj-Len Henriks-Eckerman, Riitta Jolanki (hereby appended) for a description of this phenomenon.

Additionally, the Examiner's attention is directed to amended Claims 4 and 7 *supra*. Claims 4 and 7 have been amended to recite that the ratio of reduced isoalpha acids and isoalpha acids used in the methods of the invention be in a synergistic ratio. The Applicants maintain that the unexpected results of identifying synergistic ratios and amounts of isoalpha acids and reduced isoalpha acids render the instant invention as not obvious over Tobe. As such, the Applicants respectfully request withdrawal of the rejection of Claims 4-7 over Tobe.

Conclusions

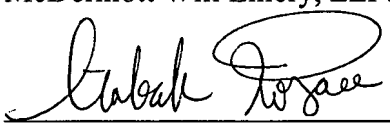
On the basis of the above remarks, this application is believed to be in condition for allowance. Accordingly, reconsideration of this application and its allowance are requested.

A request for a Three (3) Month Extension of Time, up to and including September 26, 2007 is included herewith. Pursuant to 37 C.F.R. § 1.136(a)(3), the Examiner is authorized to charge any fee under 37 C.F.R. § 1.17 applicable in the instant, as well as in future communications, to Deposit Account No.50-1133. Such an authorization should be treated as a constructive petition for extension of time in the concurrent as well as future replies.

If there are any outstanding issues that might be resolved by an interview or an Examiner's amendment, The Examiner is requested to call Applicants' agent at the telephone number shown below.

Respectfully submitted,

McDermott Will Emery, LLP.



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Attachment: Aalto-Korte et al.,Contact Dermatitis, 52(2):93-95 (2005) (3 pages).

Contact Dermatitis 2005: 52: 93–95
Printed in Denmark.

CONTACT DERMATITIS

Allergic contact dermatitis from salicyl alcohol and salicylaldehyde in aspen bark (*Populus tremula*)

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Salicyl alcohol or 2-methylolphenol is a well-known allergen in phenol-formaldehyde resins and a strong sensitizer in guinea pigs. There is 1 previous report of allergic contact dermatitis from salicyl alcohol in aspen bark. We describe a second case with concomitant allergy to salicylaldehyde. An elk researcher who had handled leaves from various trees presented with eczema of the hands, face, flexures, trunk and extremities. Patch testing showed sensitivity to salicyl alcohol, salicylaldehyde, balsam of Peru (Myroxylon pereirae resin), aspen wood dust and an extract prepared from the bark of aspen (*Populus tremula*). Weaker reactions were observed to bark extracts of rowan (*Sorbus aucuparia*), tea-leaved willow (*Salix phylicifolia*) and goat willow (*Salix caprea*). We analysed salicyl alcohol and salicylaldehyde in the bark extracts and found the 2 chemicals in equal amounts, about 0.9 μg/mg in aspen bark and in lower concentrations in rowan and the willows. We did not find either of the chemicals in the test substance of balsam of Peru (*Myroxylon pereirae*). Besides salicyl alcohol, salicylaldehyde is also recommended to be used to screen for contact allergy to aspen. Both of these chemicals should be tested in forest workers in areas where aspen is growing.

Key words: aspen, balsam of Peru, CAS 8007-00-9, CAS 90-01-7, CAS 90-02-8, *Myroxylon pereirae*, occupational, *Populus tremula*, salicyl alcohol, salicylaldehyde.

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Salicyl alcohol (2-hydroxybenzyl alcohol, 2-methylolphenol, CAS 90-01-7) is a crystalline substance that has been used as a local anaesthetic (1). It is soluble in organic solvents (e.g. alcohol, chloroform and ether) and hot water and slightly soluble in cold water. It is a well-known allergen of phenol-formaldehyde resins (2, 3). It is a strong sensitizer according to the guinea-pig maximization test (4). 1 patient with allergic contact dermatitis from salicyl alcohol in aspen bark has been diagnosed previously at Finnish Institute of Occupational Health (FIOH) (1). Salicylaldehyde (2-hydroxybenzaldehyde, CAS 90-02-8) is an oily liquid with an almond-like odour, slightly soluble in water, and it is used in perfumes and as a flavouring agent in food products (2). Trolab® (Hermal, Reinbek, Germany) perfumes and flavours series of patch test allergens includes salicylaldehyde. Salicylaldehyde and salicyl alcohol are chemically closely related, and simultaneous reactions have been reported in 5 patients with contact allergy to phenol-formaldehyde resin (2, 3).

Case Report

Our patient was a 58-year-old elk researcher with no history of atopy. In 1993, he developed eczema on his face and fingers during a field experiment in the forest, when he gathered masses of leaves of various coniferous and deciduous trees with his bare hands. The eczema spread to his flexures, trunk and extremities like atopic dermatitis but had a tendency to heal during periods of working indoors.

In 2002, he had an allergic reaction to balsam of Peru (Myroxylon pereirae resin) on patch testing in a local hospital. He had had some skin irritation from alcohol-containing deodorants but no symptoms from after-shave lotions or other cosmetic products containing fragrances.

In 2004 at FIOH, patch testing with the standard, fragrance and plant series showed allergic reactions to balsam of Peru (+) (Trolab®), salicyl alcohol (++, 2% aq. prepared from 2-methylolphenol) (Aldrich, Steinheim, Germany) and salicylaldehyde (+, 2% pet.) (Trolab®). After the positive reaction to salicyl alcohol was obtained,

we asked him to bring samples of the trees he had handled. In February, there were no leaves on the trees, hence he brought small twigs of various deciduous trees (Table 1). Ultrasonic extracts from the barks of the small twigs were prepared by extracting about 500 mg of bark (the outer dark layer and the green layer beneath the dark layer) with 10 ml of sterile water in an ultrasonic bath for 30 min (1). The extracts were left overnight at room temperature and used for patch testing on the following day. The results are summarized in Table 1. Some wood dusts (fine sanding dusts) moistened in Finn Chambers® with water were also patch tested (Table 1). Prick tests with common environmental allergens and wood dusts were negative.

On follow-up, the skin symptoms were associated with contact with broken bark of aspen and willows, sawing the same wood species and using a lawn mower in an area where aspen saplings were growing. In September 2004, the patient had vesicular eczema on his right palm after handling twigs of dark-leaved willow (*Salix myrsinifolia*) with his bare hands.

Chemical Analyses

The wood dusts and ultrasonic extracts used in the patch tests were analysed for their salicyl alcohol and salicylaldehyde content by high-performance liquid chromatography with a UV detector and an external standard method as previously described (1). Accurately weighed amounts (about 100 mg) of the wood dusts were extracted for 2 × 15 min in an ultrasonic bath containing 10 ml of distilled water at room temperature. After storage for 1 night at room temperature, the sample solution was passed through a 0.45-μm Millex-HV filter before analysis. The detection limits for salicyl alcohol and salicylaldehyde were 0.001 μg/mg and 0.003 μg/mg, respectively. The results are summarized in Table 1.

The patch test preparation of balsam of Peru (25% pet.) was analysed by gas chromatography with a mass-specific detector, and neither of the 2 substances were found (detection limit 0.01% w/w).

Discussion

Aspen leaves are the main nutrition of elk, our patient's main interest in his research work. Elk are the largest existing deer (*Alces alces*) of Europe and Asia. When the eczema appeared during his work in the forest, he had also handled many other species of trees, including rowan, alder and various willows. The ultrasonic extract of aspen

Table 1. Results of the patch tests and chemical analyses of the patch test substances, wood dusts and ultrasonic extracts of wood barks

	Ultrasonic extract of the bark			Wood dust		
Wood species	Patch test D3	Salicyl alcohol μg/ml (μg/mg bark)	Salicylaldehyde μg/ml[a] (μg/mg bark)	Patch test D5	Salicyl alcohol (μg/mg)	Salicylaldehyde (μg/mg)
Aspen, *Populus tremula*	++	42 (0.88)	44 (0.92)	+	0.27	0.087
Rowan, *Sorbus aucuparia*	+	0.17 (0.0037)	6.5 (0.14)	–	<0.005	0.025
Tea-leaved willow, *Salix phylicifolia*	+	9.3 (0.17)	<0.15 (<0.0028)	NT	ND	ND
Goat willow, *Salix caprea*	+	11 (0.18)	9.6 (0.16)	–	0.049	<0.022
Dark-leaved willow, *Salix myrsinifolia*	–	2.3 (0.042)	8.8[b] (0.16)	NT	ND	ND
Grey alder, *Alnus incana*	–	<0.05 (<0.001)	1.3 (0.030)	NT	ND	ND
Silver willow, *Salix sibirica*	NT	ND	ND	–	0.072	<0.018
Common alder, *Alnus glutinosa*	NT	ND	ND	–	<0.007	<0.020
Oak, *Quercus robur*	NT	ND	ND	–	<0.059	0.053

ND = not determined; NT = not tested.
[a]Salicylaldehyde degrades slowly in water solution, and its concentration is only indicative.
[b]The high concentration may be due to a co-eluting compound.
The corresponding concentration in the bark is given in parenthesis.

bark contained much more salicyl alcohol and salicylaldehyde than the bark extracts of the other tree species. The patch test reactions to the bark extracts were mainly in line with their content of salicyl alcohol and salicylaldehyde. Only the extract of dark-leaved willow was negative in spite of its apparent relatively high content of salicylaldehyde. On follow-up, the patient also developed skin symptoms from this species. The high salicyl alcohol content of aspen dust also explained the positive reaction to the dust.

The fresh plant material contains salicyl alcohol and salicylaldehyde in the form of glucosides such as salicin (5, 6). It is possible that there are other more important allergens in the barks of aspen and willows, e.g. these glucosides. Extracting with both hot water and ethanol would probably have yielded more salicyl alcohol and aldehyde than extracting in water at room temperature, when the chemicals are poorly soluble. Especially, salicylaldehyde may have been under-extracted in our experiment.

It is possible that the allergic reactions to salicylaldehyde and salicyl alcohol were due to cross-allergy and not due to simultaneous sensitization. The patient was exposed to both of the chemicals in equal amounts. The first aspen-allergic patient at FIOH reacted only to salicyl alcohol and not to salicylaldehyde (1), favouring the possibility of simultaneous sensitization. It is possible that the present patient was primarily sensitized to fragrances, but he had not had typical symptoms of fragrance contact allergy, and the reaction to salicyl alcohol was stronger than those to salicylaldehyde and balsam of Peru. We conclude that our patient probably had occupational allergic contact dermatitis from exposure to salicyl alcohol and salicylaldehyde in the barks of aspen, rowan and willows.

This is the second case of contact allergy from aspen bark caused by salicyl alcohol. At the time of the first case, the analysis of aspen bark by gas chromatography and mass spectrometry yielded 4 chemicals of which the allergen, salicyl alcohol, was identified by patch testing (1). The other 3 chemicals were salicylaldehyde, benzoic acid and benzyl benzoate (1). In contrast to the first patient, the present patient also reacted to salicylaldehyde. Both of the patients had positive reactions to balsam of Peru. We could not find salicyl alcohol or salicylaldehyde in the test substance of balsam of Peru. The patch tests of the present patient did not include benzoic acid or benzyl benzoate, the common constituents of aspen bark and balsam of Peru (7). These rare sensitizers were negative on patch testing in the first case (1).

Besides salicyl alcohol, salicylaldehyde is also recommended to be used to screen for contact allergy to aspen bark. Thus, it should be added to the patch test series of forest workers in areas where aspen is growing. These contact allergens are also found in rowan and willows, although in lower concentrations than in aspen.

References

1. Jolanki R, Suhonen R, Henriks-Eckerman M L, Estlander T, Kanerva L. Contact allergy to salicylic alcohol in aspen bark. *Contact Dermatitis* 1997: 37: 304–305.
2. Malten K E, Seutter E. Phenolformaldehyde resin in paper. *Contact Dermatitis* 1984: 11: 127–128.
3. Bruze M, Zimerson E. Cross-reaction patterns in patients with contact allergy to simple methylol phenols. *Contact Dermatitis* 1997: 37: 82–86.
4. Bruze M. Sensitizing capacity of 2-methylol phenol, 4-methylol phenol and 2,4,6-trimethylol phenol in the guinea pig. *Contact Dermatitis* 1986: 14: 32–38.
5. Zenk MH. Pathways of salicyl alcohol and salicin formation in *Salix purpurea* L. *Phytochemistry* 1967: 6: 245–252.
6. Julkunen-Tiitto R. Phenolic constituents of *Salix*: a chemotaxonomic survey of further Finnish species. *Phytochemistry* 1989: 28: 2115–2125.
7. Hjorth N. Eczematous allergy to balsams. *Allied Perfumes and Flavouring Agents*. Copenhagen: Munksgaard, 1961: 94–96.

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